Nicotinamide and Nicotinic Acid in Color Preservation of Fresh Meat

SUMMARY—Nicotinamide (NAm) protected the metmyoglobin (MetMb), reducing activity and oxygen consumption of ground beef or pork on refrigeration or freezer storage. Nicotinic acid (NA) increased MetMb in aerobically refrigerated ground meats, while NAm, particularly in combination with ascorbic acid, decreased it. In model systems and meats, hemochrome formation occurred with reduced myoglobin and either NA or NAm, but more readily with NA and at lower pH values. No hemochrome was formed in aerobically stored meats with NA or NAm, even with ascorbic acid present. Anaerobic conditions gave hemochromes, but only at the higher concentrations and lower pHs tried. The implication of these findings to color protection in fresh meats is discussed.

INTRODUCTION

NIACIN, OR nicotinic acid (NA), has been patented for use as a preservative of the red color in meats. Coleman et al. (1949, 1951) describe the formation of a red reaction product of NA and myoglobin (Mb). The amount recommended is 0.3 g/lb of meat. It was used commercially at one-tenth of this level. Side effects (general vasodilatation with flushing and itching) result from ingestion of 50 to 100 mg NA. Nicotinamide (NAm), on the other hand, does not produce such side effects (Press and Yaeger, 1962). NAm would therefore be preferable to NA for use in meats, provided it gives comparable color protection. At present, neither NA or NAm is allowed in meats subject to federal regulations (Bennett, 1968).

Coleman et al. (1951) do not identify the red reaction product. It may be presumed to be a hemochrome, possibly a mixed hemochrome as described by Lemberg and Legge (1949) with NA attached to one side of the porphyrin iron and globin to the other. NAm as well as NA forms hemochromes. Olcott and Lukton (1961), working with pure heme in model systems, reported hemochrome formation at lower concentrations of NAm than of other bases tried. Brown and Tappel (1957) ascribed pink pigments of canned tuna to hemochrome formation with NAm derived from nicotinamide adenine dinucleotide (NAD) during the heat treatment. Koizumi and Matsuma (1967) concluded that the pink color of cooked tuna could be improved by adding nitrogenous bases, e.g., NAm, and reducing agents to the meat, thus increasing ferrohemochrome formation. No direct comparisons of hemochrome formation with NAm versus NA in meat, or under conditions similar to those in meat, could be located in the literature.

The heme compound must be in the reduced form to give hemochromes with NA or NAm. Coleman et al. (1951) recommended that a reducing agent such as ascorbate be used in combination with the NA. Under anaerobic conditions, the meat itself may bring about the necessary reduction of metmyoglobin (MetMb) through NAD mediated enzyme systems (Watts et al., 1966). NAm, but not NA, protects the NAD in tissues from destruction by nucleosidase (Severin et al., 1963).

In view of these properties, NAm was felt to merit further study in meats. This paper reports enzymatic reduction of MetMb in ground meats stored with NAm, and compares hemochrome formation with the acid and the amide, both in model systems and in meat.

METHODS

Preparation of the meat, measurement of its MetMb reduction capacity and the rate of oxygen consumption in the meat slurries were described by Watts et al. (1966). The concentrations of added NA and NAm are expressed as mg %, i.e., mg per 100 g meat. The appropriate amount to be added to 50 g of meat was contained in 1 ml water. The controls had 1 ml water added. The additions of NAm for reduction protection studies were made immediately after grinding.

To study hemochrome formation in model systems, horse MetMb (Nutritional Biochemicals) in M/10 phosphate buffer was reduced with sodium hyposulfite. Hemochrome formation as a function of NAm or NA concentration, time and pH was evaluated by the ratio of absorbances at 530/572 mμ.

In storage studies with refrigerated meats, 3 mg % chlortetracycline was used as a preservative, and the meat in 50 g balls was held in air permeable bags at 4°C and analyzed for surface MetMb as described by Hutchins et al. (1967). Freezer storage was at −12°C. When it was desired to pack anaerobically, the meat was flushed with nitrogen in an airtight bag and test substances (ascorbic acid, NA or NAm) injected through the bag as described by Watts et al. (1966). Hemochrome formation in the stored meats was followed by changes in the K/S ratio at 530/572 mμ.

RESULTS

Effect of NAm on reducing activity of refrigerated and frozen meats

Stewart et al. (1965) had shown a decrease of the enzymatic reducing activity on refrigeration storage of ground meat, but had not worked with frozen meat. Greater decreases occur on freezing, even for a day (Table 1). The losses on freezing as with refrigeration can be restored by the addition of NAD after thawing (Fig. 1).

Table 1—Loss of reducing activity on freezer storage

Days frozen	% MetMb Reduced [1]	% MetMb Reduced [2]
Fresh	43	100
1	17	24
7	10	2

[1] Rib eye of beef, pH 5.7, 0.1% ferricyanide, 60 min.
[2] Pork ham, pH 6.1, 0.1% ferricyanide, 15 min.

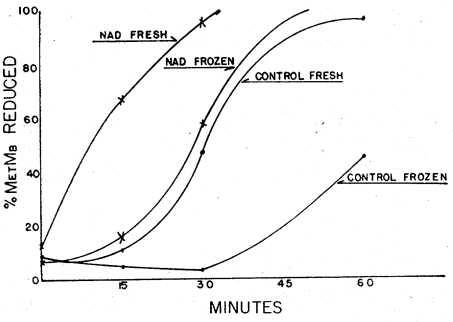

Fig. 1—Effect of NAD on reducing activity of frozen meats. Ground pork frozen 12 days, pH 6.1, 20.8 mg % NAD, 0.2 % ferricyanide.

NAm added to the ground meat before refrigeration or freezing at least partially protected the MetMb reducing activity. In 14 samples of refrigerated pork and beef and one frozen pork sample, NAm treated samples always showed greater reducing activity after storage than controls. Loss of fresh meat reducing activity averaged 68% in control samples after 4 to 6 days of refrigeration or 1 day of freezing. Table 2 gives the average losses in refrigerated ground meat treated with NAm. Concentrations as low as 6 mg % gave some protection, and there appeared to be no advantage in using concentrations greater than 60 mg %. Oxygen utilization of slurries prepared from NAm treated ground pork after refrigeration or freezing was also much higher than controls without NAm.

Table 2—NAm protection of MetMb reducing activity in refrigerated pork

Treatment[1]	No. of samples	Average reduction loss, %	Range	Standard deviation
Control	8	68	50–85	12
6 mg % NAm	5	40	35–42	4
60 or 300 mg % NAm	5	11	0–22	11

[1] Refrigeration at 4°C for 4 to 6 days.

Hemochrome formation in model systems

The spectra obtained from reduced Mb, before and after treatment with NA and NAm, respectively, are shown in Fig. 2. The spectra of the two hemochromes are quite similar, but it should be noted that a much higher concentration of NAm than NA was required to produce comparable hemochrome formation. With concentrations of 0.07 *M* NA and 0.28 *M* NAm, the hemochromes formed were nearly the same reddish-pink. With 0.07 *M* NAm, the color was purplish-red.

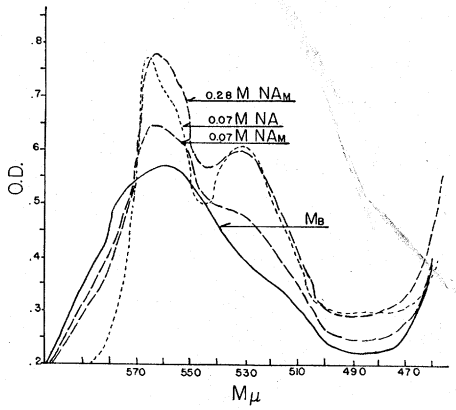

Fig. 2—Hemochrome spectra in model systems. Reduced Mb, pH 5.0 (6.7 × 10)$^{-5}$M).

All three pigments are isosbestic at 572 mμ. When Mb was titrated with NA, the spectrum developed four distinct bands which on further titration reverted to the two-banded spectrum of ordinary hemochrome. Keilin (1966) noted the formation of four-banded spectra under similar conditions and ascribed them to asymmetry of the heme iron-ligand bond caused by spatial restriction of the ligand. Keilin also found that excess NA reverted the spectrum to the two bands of ordinary hemochrome, denoting displacement of globin.

The two ligands differed with respect to the time required for hemochrome formation. With NA, the reaction was practically complete within a minute, whereas NAm continued to react over a period of hours and precipitation occurred before equilibrium was attained. Precipitation was especially rapid at high concentrations of ligand and low pH values, where most hemochrome formation occurred. Thus, a titration for complete hemochrome formation was not possible with NAm.

The effect of increasing concentrations of NA on the ratio of absorbances at 530/572 mμ at pH 5.6 are shown in Fig. 3. The ratio for zero hemochrome formation (100 % Mb) was 0.74. To determine the limiting ratio for complete hemochrome formation and the dissociation constant of the hemochrome formed, these data were treated according to the method of George et al. (1961). The limiting absorbance ratio for 100% hemochrome formation was found to be 1.63, and the dissociation constant at this pH was 0.072. Standard deviation of the values from the straight line was 4%.

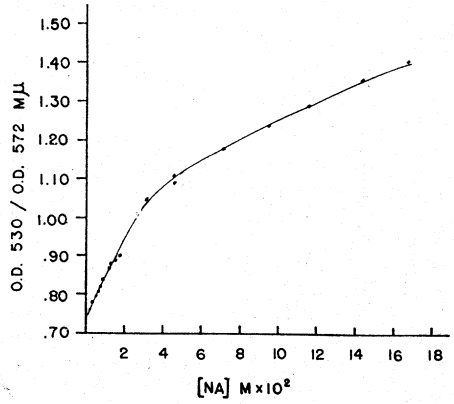

Fig. 3—Increase in the ratio of optical densities 530/572 mμ of Mb upon addition of NA at pH 5.6. Reduced Mb (3 × 10^{-5}M). Ratios were corrected for slight volume change.

Assuming the same limiting value (1.63) for the NAm as for the NA hemochrome, the effect of pH on hemochrome formation with both NA and NAm is shown in Table 3. The dissociation constants for the NAm Mb are only an approximation, since it is questionable that the reaction had reached equilibrium at 1 hr. Keilin (1966) gave a value of 0.43 for the dissociation constant of NA Mb at a pH between 6 and 7. The pH range explored (Table 3) is that of normal meat. Obviously, pH is an important factor in hemochrome formation with either ligand. Above pH 6.0 little formation occurred.

Table 3—Variation of Hemochrome Formation with pH[1]

pH	O.D. 530 mμ[2] / O.D. 572 mμ	% Hemochrome	Dissociation constants (*M*)
NA			
5.4	1.20	52.0	0.062
5.7	1.08	38.5	0.11
5.9	1.03	33.0	0.14
6.1	0.89	17.5	0.32
6.4	0.84	11.5	0.52
NAm			
5.4	0.91	19.5	0.28
5.7	0.82	9.5	0.64
5.9	0.78	5.0	1.27
6.1	0.77	3.5	1.85
6.4	0.77	3.5	1.85

[1] Reduced Mb(2.7 × 10^{-5}*M*), NA or NAm (0.067 *M*), 1 hr.
[2] Ratio for reduced Mb, 0.74.

Analysis of surface pigments of ground meats treated with NA or NAm

Aerobic storage. No hemochrome formation occurred with either ligand, even with concentrations as high as 300 mg %, when the meat was stored aerobically, with an O_2 permeable film. MetMb increased in all samples during storage. Meat containing more than one-third MetMb was visibly inferior. Concentrations of 6 mg % of either ligand had no consistent effect on MetMb formation. Higher concentrations of NAm (60 to 300 mg %) slightly retarded MetMb

formation in most samples tested, whereas the same concentrations of NA invariably accelerated pigment oxidation.

Ascorbic acid, in the amount of 11 mg % recommended by Coleman et al. (1951), decreased MetMb in 10 different samples of meat, and combinations of ascorbic acid and NAm gave better protection than either alone. Ascorbic acid added to the freshly ground meat reduced the MetMb present and gave a bright red surface (MbO_2). Even with NA, the bright red pigment was MbO_2, not hemochrome (Table 4). The effects noted cannot be attributed to pH changes; whenever necessary, the pH of additives was adjusted to that of the meat.

Table 4—Percentage of MetMb in the surface pigment of ground meat

Sample[1] treatment	% MetMb at storage indicated		
	Fresh	1 day	6 days
I. Control[2]	17	23	47
11 mg % ascorbate	0	6	33
150 mg % NA + ascorbate	0	5	61
150 mg % NAm + ascorbate	1	0	30
			3 days
II. Control[3]	3	24	33
NAm 6 mg %	5	30	39
60 mg %	0	23	31
300 mg %	0	18	28
NA 6 mg %	2	22	48
60 mg %	0	36	65
300 mg %	6	54	70

[1] Refrigerated aerobically at 4°C.
[2] Ground beef, pH 5.7.
[3] Ground pork, pH 5.8.

Anaerobic storage. There was no significant hemochrome formation with NA below 60 mg %, or NAm below 150 mg %. At higher concentrations, partial hemochrome formation was evident with both ligands. The ratios increased with time. For a given concentration, higher ratios were always obtained with NA than with NAm, as might be expected from the results with model systems. Ascorbic acid did not increase hemochrome formation under these conditions (Table 5). Pork begins to assume a reddish-pink color and beef a deeper red with ratios above 0.80. The pH of most locally purchased meat was 5.7 or above, and at these pHs the amount of hemochrome formed with NAm was not visibly detectable. However, there was visible formation of this hemochrome in meat at pH 5.3.

Table 5—Hemochrome formation with Nam or NA and ascorbate acid in anaerobic beef[1]

Sample	K/S 530/572 mμ		
	1 day	10 days	13 days
Control	0.72	0.75	0.72
11 mg % Ascorbic acid	0.75	0.75	0.72
60 mg % NAm + Ascorbic Acid	0.76	0.75	
100 mg % NAm + Ascorbic Acid	0.78	0.77	0.75
100 mg % NA + Ascorbic Acid	0.84	0.94	0.90
100 mg % NA	0.89	0.92	0.95

[1] Refrigerated at 4°C, pH 5.7.

DISCUSSION

There seems to be no justification for the addition of NA to meats. This additive definitely accelerates MetMb formation if the meat is exposed to air. Although it forms hemochromes more readily than NAm, the concentrations required to give a noticeable reddening, even with meat of low pH, would be expected to have an adverse physiological effect.

The case for NAm is somewhat stronger. The fact that it retards loss of reducing activity in stored meats, presumably by protecting NAD, could prove useful under some conditions. Bailey et al. (1964) noted that the meat pigments of hams cured at 38°F for 6 days were more stable to light in the presence of NAm and ascorbate than were those treated either with ascorbate alone or with a mixture of ascorbate and NA. They suggested a protective effect on NAD.

The unattractive purple color of meat stored anaerobically may be improved by the addition of NAm, but only if the NAm is used in concentrations of 60 mg % or more and the pH of the meat is low. There seems to be no health hazard in the use of these rather high concentrations, but there may be some doubt that the improvement achieved is sufficient to warrant such treatment.

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Ms. rec'd 3/22/68; revised 7/12/68; accepted 11/21/68.

This study was supported by Agricultural Research Service, U. S. Department of Agriculture Grant No. 12-14-100-7655 (73) administered by the Eastern Utilization Research and Development Division, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118.